Plasma miR-99a and miR-100 predict liver fibrosis progression in HIV/HCV subjects

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BACKGROUND

The lack of available biomarkers to diagnose and predict different stages of liver disease, such as non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH), with a non-invasive strategy is currently one of the main challenges that clinicians are facing. Recent evidence indicates that the plasma levels of specific microRNAs (miRs) may be significantly altered in subjects with liver injury (Gjorgjieva et al 2019), including HIV infected individuals (Franco et al 2018).

METHODS

Large-scale deep sequencing analysis of small RNA expression was performed on plasma samples from 46 HIV-1/HCV co-infected subjects that did not exhibit liver fibrosis at the time of sampling. After a mean of 10.3 years, 26 of the former subjects developed liver fibrosis (F2-4) and 20 remained without signs of liver fibrosis (F0-1). Twenty one healthy uninfected donors were also analyzed (Table 1).

Table 1. Baseline clinical and biochemical characteristics of the whole study population.

	Healthy donors	HIV-1/HCV co-infected	p-value
Parameter	Median (Range) N (%)	Median (Range) N (%)	
N	21	46	-
Age, yr	37 (29-63)	38 (27-59)	0.0876
Gender, Male, N (%)	6 (29)	33 (72)	-
Alkaline Phosphatase (U/L)	54 (30-78)	66 (43-195)	0.0022
Bilirrubin, (umol/L)	8 (3.5-25.8)	10.1 (1.5-22)	0.7638
Serum Albumin, (g/L)	-	44 (37-51)	-
Creatinin, (umol/L)	69 (49-107)	82 (62-127)	<0.0001
Total protein, (g/L))	73 (66-81)	73 (61-87)	0.9382
Cholesterol, (mmol/L)	174 (116-259)	166 (73-266)	0.4903
Triglycerides, (mmol/L)	0.9 (0.3-2.4)	1.4 (0.5-7.5)	0.0015
Glucose (mmol/L)	-	4.9 (3.7-7.8)	-
ALT, (U/L)	15 (9-55)	71 (13-422)	<0.0001
AST, (U/L)	18 (10-25)	48 (20-369)	0.0008
GGT, (U/L)	-	78 (12-1949)	-
Platelet counts (x10^9)	347 (166-387)	190 (107-301)	<0.0001

Table 2. Baseline clinical and biochemical characteristics of non-progressing and progressing HIV-1/HCV co-infected subjects.

	Non-progressing (F0-1)	Progressing (F2-4)	p-value
Parameter	Median (Range) N (%)	Median (Range) N (%)	
N	20	26	-
Age, yr	39 (27-59)	36 (29-59)	0.5188
Gender, Male, N (%)	14 (70)	19 (73)	-
Alkaline Phosphatase (U/L)	66 (43-139)	70 (43-195)	0,9929
Bilirrubin, (umol/L)	8.5 (4.4-17.2)	11.6 (1.5-22)	0.0090
Serum Albumin, (g/L)	44 (39-51)	45 (37-51)	0.6420
Creatinin, (umol/L)	84 (71-120)	82 (62-127)	0.9982
Total protein, (g/L))	75 (66-84)	72 (61-87)	0.6968
Cholesterol, (mmol/L)	168 (96-232)	166 (73-266)	0.8666
Triglycerides, (mmol/L)	1.9 (0.6-7.5)	1.3 (0.5-3.4)	0.2449
Glucose (mmol/L)	4.7 (4.2-6.6)	5.1 (3.7-7.8)	0.3854
ALT, (U/L)	51 (13-422)	84 (21-268)	0.1895
AST, (U/L)	37 (20-369)	61 (20-145)	0.4548
GGT, (U/L)	63 (20-262)	81 (12-1949)	0.2345
Platelet counts (x10^9)	188 (114-301)	192 (107-297)	0.6278
FIB-4	1.1 (0.6-3.8)	1.4 (0.5-2.8)	0.2149
Steatosis	3 (15%)	6 (23%)	0.5726
CD4+	624 (336-1234)	481 (234-1431)	0.0300
HCV VL (log)	5.6 (1.7-5.9)	5.7 (1.7-7.5)	0.1718
Detectable HIV VL (>100)	6 (30%)	12 (46%)	0.4575

Plasma circulating **microRNAs** are potential biomarkers of **liver disease** progression in HIV-infected subjects.

RESULTS

At the time of sampling, there were not significant clinical differences between liver fibrosis progressing and non-progressing subjects (i.e. sex, age, AST, ALT, GGT, platelets, FIB-4, liver fibrosis) (Table 2). A total of 1355 different miRs were identified. When compared with healthy donors, HIV-1/HCV subjects showed significant (fold change> 2 and adjusted p< 0.05) dysregulated expression of 44 miRs, 38 of them upregulated (ranging from 13.8 to 2.0 fold increase) (Figure 1). Previously described circulating miRs associated with NAFLD in the general population, miR-122, miR-34a and miR-192, were also found here within the 38 upregulated miRs. Of the 38 upregulated miRs, 7 (miR-885-5p, miR-100-5p, miR-193-5p, miR-99a-5p, miR-203a-3p, miR-5588-5p and miR-99a-3p) were significantly upregulated in the 26 subjects that progressed to liver fibrosis when compared to the 20 subjects that did not progressed (p< 0.005). Two of these miRs, miR-99a-5p and miR-100-5p, were highly associated with liver fibrosis progression (p< 0.0001) and displayed a significant linear correlation with liver fibrosis values of the entire study cohort (r= 0.51, p= 0.0003 and r= 0.48, p= 0.0006, respectively) (Figure 2).

Figure 1. Expression heat map of 44 dysregulated miRNAs in baseline plasma of 46 HIV-1/HCV co-infected subjects.

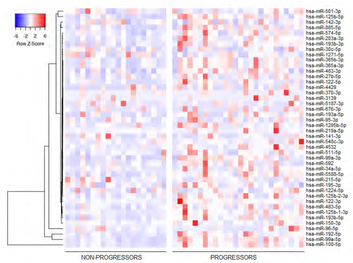
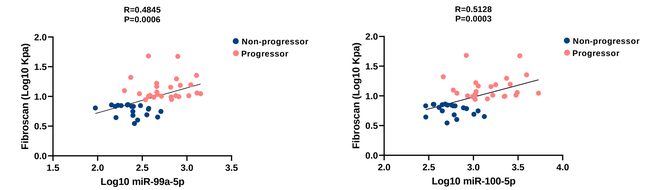


Table 2. Differences in miRNAs baseline plasma levels between non-progressing and progressing HIV-1/HCV co-infected subjects.

	Non-progressing (F0-1)	Progressing (F2-4)	p-value[1]
Years to end point	10 (4.5-16.2)	10.7 (4-13.5)	0.5726
Fibrosis[2] at end point (Kpa)	6.3 (3.5-7.3)	11.2 (8.8-48)	0.0002
miR-885-5p (baseMean)[3]	22.9 (0.8-106)	87.8 (6.0-221.1)	<0.0001
miR-100-5p (baseMean)	541.3 (292.2-1326.0)	1285.0 (460.0-5364.0)	<0.0001
miR-193b-5p (baseMean)	10.0 (0.0-90.1)	40.3 (11.6-137.4)	0.0009
miR-99a-5p (baseMean)	248.7 (94.5-512.4)	589.2 (199.5-1424.0)	<0.0001
miR-203a-3p (baseMean)	5.1 (0.0-16.7)	15.0 (0.0-81.3)	0.0021
miR-5588-5p (baseMean)	2.9 (0.0-12.9)	10.3 (0.0-35.1)	0.0002
miR-99a-3p (baseMean)	2.9 (0.0-16.4)	9.7 (0.0-23.6)	0.0003

[1]Probability that a fold change as strong as the observed one, or even stronger, would be seen under the situation described by the null hypothesis (no difference)
[2]Fibrosis stage was determined by fibroscan
[3]baseMean is the average of the normalized count values according to DeSeq2, divided by size factors, taken over all samples

Figure 2. Correlation between baseline miR-99a-5p and miR-100-5p plasma levels and end-point liver fibrosis score.



CONCLUSIONS

Circulating miR-99a-5p and miR-100-5p are significantly associated with liver fibrosis progression in subjects with HIV-1/HCV co-infections, even before liver fibrosis is detectable. This study demonstrates the potential of miRs as biomarkers in the progression of liver injury in HIV-infected subjects. Levels of miR-99a-5p and miR-100-5p may be suitable markers of liver fibrosis amelioration in HIV-1/HCV co-infected patients treated with HCV DAAs and cured of HCV infection.

REFERENCES

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ADDITIONAL INFORMATION

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